TO: _____ On (note date): MAR 2 2 2005 Pat. No.: 6825004
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SECOND REQUEST (DIFFERENT CORRECITONS), SUPERSEDE OR RECONSIDERATION
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Date Assigned: ___/___/___ Turned In: ___/___/___

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New/different correction(s) requested. Check Intranet or with RTIS. (circle one) Y / N

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(Revised 12/15/2003 cbn)

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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450 Alexandria, VA 22313 on March 11, 2005.

Frank C. Eisenschenk

Frank C. Eisenschenk, Ph.D., Patent Attorney

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 CFR 1.322
Docket No. G-046US02PCT
Patent No. 6,825,004

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Marta Blumenfeld, Lydie Bougueleret, Ilya Chumakov
Issued	:	November 30, 2004
Patent No.	:	6,825,004
For	:	Nucleic Acids Encoding Human TBC-1 Protein and Polymorphic Markers Thereof

Mail Stop Certificate of Corrections Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION
UNDER 37 CFR 1.322 (OFFICE MISTAKE)

Sir:

A Certificate of Correction (in duplicate) for the above-identified patent has been prepared and is attached hereto.

In the left-hand column below is the column and line number where errors occurred in the patent. In the right-hand column is the page and line number in the application where the correct information appears.

Patent Reads:	**Application Reads:**
Column 8, lines 16-17: "Fab, in Fab"'	Page 9, lines 21-22: --Fab, Fab'--
Column 10, line 65: "sequences ad not"	Page 12, line 26: --sequences and not--
Column 10, line 66: "conditions-under"	Page 12, line 26: --conditions under--

Column 12, line 60: "(CH_2CH_3)"	Page 15, line 1: --(CH_2CH_2)--
Column 12, line 62: "CH=CH—"	Page 15, line 2: -- —CH=CH— --
Column 14, line 1: "art Preferably"	Page 16, line 13: --art. Preferably--
Column 16, line 11: "murine tbc"	Page 18, line 29: --murine *tbc1*--
Column 16, line 14: "hematopoictic"	Page 18, line 31: --hematopoietic--
Column 17, line 32: "this fist"	Page 20, line 10: --this first--
Column 22, line 53: "SEQ ED"	Page 26, line 15: --SEQ ID--
Column 25, line 23: "401-400"	Page 29, line 16: --401-600--
Column 26, line 45: "polygonal"	Page 30, line 31: --polyclonal--
Column 29, line 45: "(1991)"	Page 34, line 15: --(1997)--
Column 31, line 10: "K Genotyping"	Page 36, line 3: --B-Genotyping--
Column 33, line 1: "in die art"	Page 38, line 10: --in the art--
Column 33, line 25: "technique/The"	Page 38, line 25: --technique. The--
Column 34, line 17: "Smimov"	Page 39, line 26: --Smirnov--
Column 34, line 58: "(Haiju el al.,"	Page 40, line 14: --(Harju et al.,--

Column 39, line 21: "WO 92110092"	Page 45, line 14: --WO 92/10092--
Column 42, line 63: "5014000"	Page 49, line 25: --5001-6000--
Column 43, line 15: "40, 35, 50"	Page 49, line 36 through to page 50, line 1: --40, 50--
Column 47, line 56: "WO 92/10692"	Page 55, line 10: --WO 92/10092--
Column 50, line 52: "PBPV"	Page 58, line 30: --pBPV--
Column 51, line 28: "include lac"	Page 59, line 25: --include lacI--
Column 52, line 57: "VR459"	Page 61, line 14: --VR-659--
Column 55, line 29: "TDC-1"	Page 64, line 12: --*TBC-1*--
Column 56, line 50: "amplified-by"	Page 65, line 33: --amplified by--
Column 59, line 22: "96well"	Page 68, line 32: --96-well--
Column 59, line 27: "The ways are"	Page 68, line 35: --The arrays are--
Column 60, line 50: "SEQ ED"	Page 70, line 19: --SEQ ID--
Column 60, line 58: "ace transferase"	Page 70, line 24: --acetyl transferase--
Column 60, line 62: "vector"	Page 70, line 27: --vectors--
Column 61, line 6: "an,exogenous"	Page 70, line 34: --an exogenous--

Column 61, line 32: "invention arm"	Page 71, line 12: --invention are--
Column 67, line 7: "of tie considered"	Page 76, line 14: --of the considered--
Column 69, line 6: "*Genet* 996"	Page 78, line 25: --*Genet* 1996--
Column 69, lines 11-12: "75: 20 805-816"	Page 78, lines 28-29: --75 : 805-816--
Column 199, line 55: "comprising 6 amino acids of SEQ ID NO:5"	Amendment dated May 10, 2004 (original claim 50, renumbered as claim 1): --comprising SEQ ID NO:5--
Column 199, line 60: "comprising 6 amino acids of SEQ ID NO:5"	Amendment dated May 10, 2004 (original claim 65, renumbered as claim 2): --comprising SEQ ID NO:5--
Column 200, line 64: "polynucleotides"	Amendment dated May 10, 2004 (original claim 80, renumbered as claim 9): --polynucleotide--
Column 200, line 66: "or e) the"	Amendment dated May 10, 2004 (original claim 80, renumbered as claim 9): --or c) the--
Column 202, line 16: "purifying of isolating"	Amendment dated May 10, 2004 (original claim 97, renumbered as claim 26): --purifying or isolating--.

A true and correct copy of pages 9, 12, 15, 16, 18, 20, 26, 29, 30, 34, 36, 38, 39, 40, 45, 49, 50, 55, 58, 59, 61, 64, 65, 68, 70, 71, 76, and 78, of the specification, as filed, and Applicants' Amendment Under 37 CFR §1.111 dated May 10, 2004 which supports Applicants' assertion of the errors on the part of the Patent Office accompanies this Certificate of Correction.

Approval of the Certificate of Correction is respectfully requested.

Respectfully submitted,

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FCE/ems
Attachments: Certificate of Correction in duplicate;
Copy of pages 9, 12, 15, 16, 18, 20, 26, 29, 30, 34, 36, 38, 39, 40, 45, 49, 50, 55, 58, 59, 61, 64, 65, 68, 70, 71, 76, and 78 of the specification
Copy of Applicants' Amendment dated May 10, 2004

The term "purified" is used herein to describe a polypeptide of the invention which has been separated from other compounds including, but not limited to nucleic acids, lipids, carbohydrates and other proteins. A polypeptide is substantially pure when at least about 50%, preferably 60 to 75% of a sample exhibits a single polypeptide sequence. A substantially pure polypeptide typically comprises about 50%, preferably 60 to 90% weight/weight of a protein sample, more usually about 95%, and preferably is over about 99% pure. Polypeptide purity or homogeneity is indicated by a number of means well known in the art, such as polyacrylamide gel electrophoresis of a sample, followed by visualizing a single polypeptide band upon staining the gel. For certain purposes higher resolution can be provided by using HPLC or other means well known in the art.

As used herein, the term "non-human animal" refers to any non-human vertebrate, birds and more usually mammals, preferably primates, farm animals such as swine, goats, sheep, donkeys, and horses, rabbits or rodents, more preferably rats or mice. As used herein, the term "animal" is used to refer to any vertebrate, preferable a mammal. Both the terms "animal" and "mammal" expressly embrace human subjects unless preceded with the term "non-human".

As used herein, the term "antibody" refers to a polypeptide or group of polypeptides which are comprised of at least one binding domain, where an antibody binding domain is formed from the folding of variable domains of an antibody molecule to form three-dimensional binding spaces with an internal surface shape and charge distribution complementary to the features of an antigenic determinant of an antigen, which allows an immunological reaction with the antigen. Antibodies include recombinant proteins comprising the binding domains, as wells as fragments, including Fab, Fab', $F(ab)_2$, and $F(ab')_2$ fragments.

As used herein, an "antigenic determinant" is the portion of an antigen molecule, in this case a TBC-1 polypeptide, that determines the specificity of the antigen-antibody reaction. An "epitope" refers to an antigenic determinant of a polypeptide. An epitope can comprise as few as 3 amino acids in a spatial conformation which is unique to the epitope. Generally an epitope consists of at least 6 such amino acids, and more usually at least 8-10 such amino acids. Methods for determining the amino acids which make up an epitope include x-ray crystallography, 2-dimensional nuclear magnetic resonance, and epitope mapping e.g. the Pepscan method described by Geysen et al. 1984; PCT Publication No. WO 84/03564; and PCT Publication No. WO 84/03506.

Throughout the present specification, the expression "nucleotide sequence" may be employed to designate indifferently a polynucleotide or an oligonucleotide or a nucleic acid. More precisely, the expression "nucleotide sequence" encompasses the nucleic material itself and is thus not restricted to the sequence information (i.e. the succession of letters chosen among the four base letters) that biochemically characterizes a specific DNA or RNA molecule.

nucleotides in length. Each biallelic marker therefore corresponds to two forms of a polynucleotide sequence included in a gene, which, when compared with one another, present a nucleotide modification at one position. Preferably, the sequences defining a biallelic marker include a polymorphic base selected from the group consisting of the biallelic markers A1 to A19 and the complements thereof. In some embodiments the sequences defining a biallelic marker comprise one of the sequences selected from the group consisting of P1 to P7, P9 to P13, P15 to P19 and the complementary sequences thereto. Likewise, the term "marker" or "biallelic marker" requires that the sequence is of sufficient length to practically (although not necessarily unambiguously) identify the polymorphic allele, which usually implies a length of at least 4, 5, 6, 10, 15, 20, 25, or 40 nucleotides.

The term "upstream" is used herein to refer to a location which is toward the 5' end of the polynucleotide from a specific reference point.

The terms "base paired" and "Watson & Crick base paired" are used interchangeably herein to refer to nucleotides which can be hydrogen bonded to one another be virtue of their sequence identities in a manner like that found in double-helical DNA with thymine or uracil residues linked to adenine residues by two hydrogen bonds and cytosine and guanine residues linked by three hydrogen bonds (See Stryer, L., *Biochemistry,* 4th edition, 1995).

The terms "complementary" or "complement thereof" are used herein to refer to the sequences of polynucleotides which is capable of forming Watson & Crick base pairing with another specified polynucleotide throughout the entirety of the complementary region. For the purpose of the present invention, a first polynucleotide is deemed to be complementary to a second polynucleotide when each base in the first polynucleotide is paired with its complementary base. Complementary bases are, generally, A and T (or A and U), or C and G. "Complement" is used herein as a synonym from "complementary polynucleotide", "complementary nucleic acid" and "complementary nucleotide sequence". These terms are applied to pairs of polynucleotides based solely upon their sequences and not any particular set of conditions under which the two polynucleotides would actually bind.

Variants and fragments

1. Polynucleotides

The invention also relates to variants and fragments of the polynucleotides described herein, particularly of a *TBC-1* gene containing one or more biallelic markers according to the invention.

Variants of polynucleotides, as the term is used herein, are polynucleotides that differ from a reference polynucleotide. A variant of a polynucleotide may be a naturally occurring variant such as a naturally occurring allelic variant, or it may be a variant that is not known to occur naturally. Such non-naturally occurring variants of the polynucleotide may be made by mutagenesis techniques, including those applied to polynucleotides, cells or organisms. Generally, differences

thiomethylene bond, a (CH_2CH_2) carba bond, a (CO-CH_2) cetomethylene bond, a (CHOH-CH_2) hydroxyethylene bond), a (N-N) bound, a E-alcene bond or also a -CH=CH- bond.

The polypeptide accoding to the invention could have post-translational modifications. For example, it can present the following modifications: acylation, disulfide bond formation, prenylation, carboxymethylation and phosphorylation.

A polypeptide fragment is a polypeptide having a sequence that entirely is the same as part but not all of a given polypeptide sequence, preferably a polypeptide encoded by a *TBC-1* gene and variants thereof. Preferred fragments include those regions possessing antigenic properties and which can be used to raise antibodies against the TBC-1 protein.

Such fragments may be "free-standing", i.e. not part of or fused to other polypeptides, or they may be comprised within a single larger polypeptide of which they form a part or region. However, several fragments may be comprised within a single larger polypeptide.

As representative examples of polypeptide fragments of the invention, there may be mentioned those which comprise at least about 5, 6, 7, 8, 9 or 10 to 15, 10 to 20, 15 to 40, or 30 to 55 amino acids of the TBC-1. In some embodiments, the fragments contain at least one amino acid mutation in the TBC-1 protein.

Identity Between Nucleic Acids Or Polypeptides

The terms "percentage of sequence identity" and "percentage homology" are used interchangeably herein to refer to comparisons among polynucleotides and polypeptides, and are determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity. Homology is evaluated using any of the variety of sequence comparison algorithms and programs known in the art. Such algorithms and programs include, but are by no means limited to, TBLASTN, BLASTP, FASTA, TFASTA, and CLUSTALW (Pearson and Lipman, 1988; Altschul et al., 1990; Thompson et al., 1994; Higgins et al., 1996; Altschul et al., 1993). In a particularly preferred embodiment, protein and nucleic acid sequence homologies are evaluated using the Basic Local Alignment Search Tool ("BLAST") which is well known in the art (see, e.g., Karlin and Altschul, 1990; Altschul et al., 1990, 1993, 1997). In particular, five specific BLAST programs are used to perform the following task:

(1) BLASTP and BLAST3 compare an amino acid query sequence against a protein sequence database;

(2) BLASTN compares a nucleotide query sequence against a nucleotide sequence database;

(3) BLASTX compares the six-frame conceptual translation products of a query nucleotide sequence (both strands) against a protein sequence database;

(4) TBLASTN compares a query protein sequence against a nucleotide sequence database translated in all six reading frames (both strands); and

(5) TBLASTX compares the six-frame translations of a nucleotide query sequence against the six-frame translations of a nucleotide sequence database.

The BLAST programs identify homologous sequences by identifying similar segments, which are referred to herein as "high-scoring segment pairs," between a query amino or nucleic acid sequence and a test sequence which is preferably obtained from a protein or nucleic acid sequence database. High-scoring segment pairs are preferably identified (i.e., aligned) by means of a scoring matrix, many of which are known in the art. Preferably, the scoring matrix used is the BLOSUM62 matrix (Gonnet et al., 1992; Henikoff and Henikoff, 1993). Less preferably, the PAM or PAM250 matrices may also be used (see, e.g., Schwartz and Dayhoff, eds., 1978). The BLAST programs evaluate the statistical significance of all high-scoring segment pairs identified, and preferably selects those segments which satisfy a user-specified threshold of significance, such as a user-specified percent homology. Preferably, the statistical significance of a high-scoring segment pair is evaluated using the statistical significance formula of Karlin (see, e.g., Karlin and Altschul, 1990).

Stringent Hybridization Conditions

By way of example and not limitation, procedures using conditions of high stringency are as follows: Prehybridization of filters containing DNA is carried out for 8 h to overnight at 65°C in buffer composed of 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 µg/ml denatured salmon sperm DNA. Filters are hybridized for 48 h at 65°C, the preferred hybridization temperature, in prehybridization mixture containing 100 µg/ml denatured salmon sperm DNA and 5-20 X 10^6 cpm of ^{32}P-labeled probe. Alternatively, the hybridization step can be performed at 65°C in the presence of SSC buffer, 1 x SSC corresponding to 0.15M NaCl and 0.05 M Na citrate. Subsequently, filter washes can be done at 37°C for 1 h in a solution containing 2 x SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA, followed by a wash in 0.1 X SSC at 50°C for 45 min. Alternatively, filter washes can be performed in a solution containing 2 x SSC and 0.1% SDS, or 0.5 x SSC and 0.1% SDS, or 0.1 x SSC and 0.1% SDS at 68°C for 15 minute intervals. Following the wash steps, the hybridized probes are detectable by autoradiography. Other conditions of high stringency which may be used are well known in the art and as cited in Sambrook et al., 1989; and Ausubel et al., 1989, are incorporated herein in their entirety. These hybridization conditions are suitable for a nucleic acid molecule of about 20 nucleotides in length. There is no need to say that the hybridization conditions described above are

Homologies Of The Novel Human Gene Translation Product With A Known Murine Protein.

A novel human gene was found in this candidate region. It presents a good probability to be involved in cancer. Database homology searches have allowed the inventors to determine that the translation product of this novel human gene has significant identity with a murine protein called tbc1. Therefore, the novel human gene of the invention has thus been called *TBC-1* throughout the present specification. *TBC-1* comprises an open Reading frame that encodes a novel protein, the TBC-1 protein. Based on sequence similarity, an alignment of a portion of the TBC-1 amino acid sequence with the known tbc1 murine protein, it is expected that TBC1 protein may play a role in the cell cycle and in differentiation of various tissues. Indeed, the TBC1 protein contains a 200 amino acid domain called the TBC domain that is homologous to regions in the tre2-oncogene and in the yeast regulators of mitosis BUB2 and cdc16.

The cDNA of the murine *tbc1* gene has been described in US Patent No US 5,700,927 and it encodes a putative protein product of 1141 amino acids. The N-terminus of the murine tbc1 protein contains stretches of cysteines and histidines which may form zinc finger structures in the mature polypeptides. The N-terminus also comprises short stretches of basic amino acids which may be involved in a nuclear localization signal. The TBC domain of the murine tbc1 protein contains several tyrosine residues which are conserved in BUB2 and cdc16. The C-terminus of the murine tbc1 protein contains a long stretch of evenly spaced leucine residues which are susceptible to form a leucine zipper motif.

The murine *tbc1* gene has been shown to be highly expressed in testis and kidney. However, lower levels of expression have also be identified in lung, spleen, brain, and heart. Moreover, murine tbc1 is a nuclear protein which is expressed in a cell- and stage-specific manner.

Studies of murine bone marrow have demonstrated that erythroid cells and megakaryocytes expressed substantial levels of the murine tbc1 protein, but none was detected in mature neutrophils. Similarly, spermatogonia do not express murine *tbc1*, but primary and secondary spermatocytes express abundant *tbc1*. Later in the differentiation of the germ cells, the *tbc1* levels appear to decrease in spermatids and active sperm. The differentiation program of spermatogonia to spermatocytes therefore involves a significant upregulation of murine *tbc1* expression.

The general distribution of murine *tbc1* is not tissue-specific, but is cell-specific within individual tissues and intimately linked to tissue differentiation. The developmental expression of murine *tbc1*, particularly in hematopoietic and germ cells, suggests that this gene plays a role in the terminal differentiation program of several tissues.

Consequently, an alteration in the expression of the *TBC-1* gene or in the amino acid sequence of the TBC-1 protein leading to an altered biological activity of the latter is likely to cause, directly or indirectly, cell proliferation disorders and thus diseases related to an abnormal cell proliferation such as cancer, particularly prostate cancer.

The *TBC-1* introns defined hereinafter for the purpose of the present invention are not exactly what is generally understood as "introns" by the one skilled in the art and will consequently be further defined below.

Generally, an intron is defined as a nucleotide sequence that is present both in the genomic DNA and in the unspliced mRNA molecule, and which is absent from the mRNA molecule which has already gone through splicing events. In the case of the *TBC-1* gene, the inventors have found that at least two different spliced mRNA molecules are produced when this gene is transcribed, as it will be described in detail in a further section of the specification. The first spliced mRNA molecule comprises Exons 1 and 2. Thus, the genomic nucleotide sequence comprised between Exon 1 and Exon 2 is an intronic sequence as regards to this first mRNA molecule, despite the fact that this intronic sequence contains Exon 1*bis*. In contrast, Exon 1*bis* is of course an exonic nucleotide sequence as regards to the second *TBC-1* mRNA molecule.

For the purpose of the present invention and in order to make a clear and unambiguous designation of the different nucleic acids encompassed, it has been postulated that the polynucleotides contained both in any of the nucleotide sequences of SEQ ID Nos 1 or 2 and in any of the nucleotide sequences of SEQ ID Nos 3 or 4 are considered as exonic sequences. Conversely, the polynucleotides contained in any of the nucleotide sequences of SEQ ID Nos 1 or 2 but which are absent both from the nucleotide sequence of SEQ ID No 3 and from the nucleotide sequence of SEQ ID No 4 are considered as intronic sequences.

The nucleic acids defining the *TBC-1* introns described above, as well as their fragments and variants, may be used as oligonucleotide primers or probes in order to detect the presence of a copy of the *TBC-1* gene in a test sample, or alternatively in order to amplify a target nucleotide sequence within the *TBC-1* intronic sequences.

Thus, the invention embodies purified, isolated, or recombinant polynucleotides comprising a nucleotide sequence selected from the group consisting of the 15 exons of the *TBC-1* gene which are described in the present invention, or a sequence complementary thereto. The invention also deals with purified, isolated, or recombinant nucleic acids comprising a combination of at least two exons of the *TBC-1* gene, wherein the polynucleotides are arranged within the nucleic acid, from the 5'-end to the 3'-end of said nucleic acid, in the same order as in SEQ ID Nos 1 and 2.

Thus, the invention embodies purified, isolated, or recombinant polynucleotides comprising a nucleotide sequence selected from the group consisting of the introns of the *TBC-1* gene, or a sequence complementary thereto.

The invention also encompasses a purified, isolated, or recombinant polynucleotide comprising a nucleotide sequence having at least 70, 75, 80, 85, 90, or 95% nucleotide identity with a sequence selected from the group consisting of SEQ ID Nos 1 and 2 or a complementary sequence thereto or a fragment thereof. The nucleotide differences as regards to the nucleotide sequence of SEQ ID Nos 1 or 2 may be generally randomly distributed throughout the entire nucleic acid.

polynucleotide selected from the group consisting of the 5' and 3' regulatory regions, or a sequence complementary thereto or a variant thereof or a biologically active fragment thereof.

Another object of the invention consists of purified, isolated or recombinant nucleic acids comprising a polynucleotide that hybridizes, under the stringent hybridization conditions defined herein, with a polynucleotide selected from the group consisting of the nucleotide sequences of the 5'- and 3' regulatory regions, or a sequence complementary thereto or a variant thereof or a biologically active fragment thereof.

The 5'UTR and 3'UTR regions of a gene are of particular importance in that they often comprise regulatory elements which can play a role in providing appropriate expression levels, particularly through the control of mRNA stability.

A 5' regulatory polynucleotide of the invention may include the 5'-UTR located between the nucleotide at position 1 and the nucleotide at position 170 of SEQ ID No 3, or a biologically active fragment or variant thereof.

Alternatively, a 5'-regulatory polynucleotide of the invention may include the 5'-UTR located between the nucleotide at position 1 and the nucleotide at position 175 of SEQ ID No 4, or a biologically active fragment or variant thereof.

A 3' regulatory polynucleotide of the invention may include the 3'-UTR located between the nucleotide at position 3726 and the nucleotide at position 3983 of SEQ ID No 4, or a biologically active fragment or variant thereof.

Thus, the invention also pertains to a purified or isolated nucleic acid which is selected from the group consisting of :

a) a nucleic acid comprising the nucleotide sequence of the 5' regulatory region;

b) a nucleic acid comprising a biologically active fragment or variant of the nucleic acid of the 5' regulatory region.

Preferred fragments of the nucleic acid of the 5' regulatory region have a length of about 1000 nucleotides, more particularly of about 400 nucleotides, more preferably of about 200 nucleotides and most preferably about 100 nucleotides. More particularly, the invention further includes specific elements within this regulatory region, these elements preferably including the promoter region.

Preferred fragments of the 3' regulatory region are at least 50, 100, 150, 200, 300 or 400 bases in length.

By a "biologically active fragment or variant" of a *TBC-1* regulatory polynucleotide according to the present invention is intended a polynucleotide comprising or alternatively consisting in a fragment of said polynucleotide which is functional as a regulatory region for expressing a recombinant polypeptide or a recombinant polynucleotide in a recombinant cell host.

For the purpose of the invention, a nucleic acid or polynucleotide is "functional" as a regulatory region for expressing a recombinant polypeptide or a recombinant polynucleotide if said

The polypeptides or peptides thus obtained may be purified, for example by high performance liquid chromatography, such as reverse phase and/or cationic exchange HPLC, as described by Rougeot et al. (1994). The reason to prefer this kind of peptide or protein purification is the lack of byproducts found in the elution samples which renders the resultant purified protein or peptide more suitable for a therapeutic use.

Another object of the present invention consists in a purified or isolated TBC-1 polypeptide or a fragment or a variant thereof.

In a preferred embodiment, the TBC-1 polypeptide comprises an amino acid sequence of SEQ ID No 5 or a fragment or a variant thereof. The present invention also embodies isolated, purified, and recombinant polypeptides comprising a contiguous span of at least 6 amino acids, preferably at least 8 to 10 amino acids, more preferably at least 12, 15, 20, 25, 30, 40, 50, 100, 150 or 200 amino acids of SEQ ID No 5. The present invention also embodies isolated, purified, and recombinant polypeptides comprising a contiguous span of at least 6 amino acids, preferably at least 8 to 10 amino acids, more preferably at least 12, 15, 20, 25, 30, 40, 50, 100, 150 or 200 amino acids of SEQ ID No 5, wherein said contiguous span includes at least 1, 2, 3, 5 or 10 of the following amino acid positions: 1-200, 201-400, 401-600, 601-800, 801-1000, 1001-1168.

The invention also encompasses a purified, isolated, or recombinant polypeptides comprising an amino acid sequence having at least 90, 95, 98 or 99% amino acid identity with the amino acid sequence of SEQ ID No 5 or a fragment thereof.

The TBC-1 polypeptide of the invention possesses amino acid homologies as regards to the murine TBC-1 protein of 1141 amino acids in length which is described in US Patent No US 5,700,927. The TBC-1 protein of the invention also possesses some homologies with two other proteins : the Pollux drosophila protein (Zhang et al., 1996) and the CDC16 protein from *Caenorhabditis elegans* (Wilson et al., 1994). Figure 1 represents an amino acid alignment of a portion of the amino acid sequence of the TBC-1 protein of SEQ ID No 5 with other proteins sharing amino acid homology with TBC-1. The upper line shows the whole amino acid sequence of the murine tbc-1 protein described in US Patent No US 5,700,927; the second line represents part of the amino acid sequence of the TBC-1 protein of SEQ ID No 5; the third line (Genbank access No : *dmu50542*) depicts the amino acid sequence of the Pollux protein mentioned above; the fourth line (Genbank access No : *celf35h12*) shows the amino acid sequence of the *C. elegans* protein mentioned above; the fifth line presents positions in which consensus amino acids are identified, i.e. amino acids shared by the sequences presented in the four upper lines, when present.

The TBC-1 polypeptide of the amino acid sequence of SEQ ID No 5 has 1168 amino acids in length. The TBC-1 polypeptide includes a "TBC domain" which is spanning from the amino acid in position 786 to the amino acid in position 974 of the amino acid sequence of SEQ ID No 5. This TBC domain is represented in Figure 1 as a grey area spanning from the amino acid numbered 758 to the amino acid numbered 949. This TBC domain is likely to regulate protein-protein interactions.

Moreover, the TBC-1 TBC domain includes the amino acid sequence EVGYCQGL, spanning from the amino acid in position 886 to the amino acid in position 893 of the amino acid sequence of SEQ ID No 5. The EVGYCQGL amino acid sequence spans from the amino acid numbered 861 to the amino acid numbered 868 of Figure 1. This site may interact with a kinase. Based on the structural similarity to *cdc16*, a yeast regulator of mitosis, TBC-1 is likely to regulate mitosis and cytokinesis by interacting with other proteins which also participate with the regulation of mitosis, cytokinesis and septum formation.

Preferred polypeptides of the invention comprise the TBC domain of TBC-1, or alternatively at least the EVGYCQGL amino acid sequence motif.

A further object of the present invention concerns a purified or isolated polypeptide which is encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID Nos 1, 2, 3, and 4 or fragments or variants thereof.

A single variant molecule of the TBC-1 protein is explicitly excluded from the scope of the present invention, which is a polypeptide having the same amino acid sequence than the murine tbc1 protein described in the US Patent No 5,700,927.

Amino acid deletions, additions or substitutions in the TBC-1 protein are preferably located outside of the TBC domain as defined above. Most preferably, a mutated TBC-1 protein has an intact "EVGYCQGL" amino acid motif.

Such a mutated TBC-1 protein may be the target of diagnostic tools, such as specific monoclonal or polyclonal antibodies, useful for detecting the mutated TBC-1 protein in a sample.

The invention also encompasses a TBC-1 polypeptide or a fragment or a variant thereof in which at least one peptide bound has been modified as described in the "Definitions" section.

Antibodies That Bind *TBC-1* Polypeptides of the Invention

Any TBC-1 polypeptide or whole protein may be used to generate antibodies capable of specifically binding to an expressed TBC-1 protein or fragments thereof as described.

One antibody composition of the invention is capable of specifically binding or specifically bind to the variant of the TBC-1 protein of SEQ ID No 5. For an antibody composition to specifically bind to TBC-1, it must demonstrate at least a 5%, 10%, 15%, 20%, 25%, 50%, or 100% greater binding affinity for TBC-1 protein than for another protein in an ELISA, RIA, or other antibody-based binding assay.

In a preferred embodiment, the invention concerns antibody compositions, either polyclonal or monoclonal, capable of selectively binding, or selectively bind to an epitope-containing a polypeptide comprising a contiguous span of at least 6 amino acids, preferably at least 8 to 10 amino acids, more preferably at least 12, 15, 20, 25, 30, 40, 50, 100, 150 or 200 amino acids of SEQ ID No 5; Optionally said epitope comprises at least 1, 2, 3, 5 or 10 of the following amino acid positions : 1-200, 201-400, 401-600, 601-800, 801-1000, 1001-1168.

hybridize to the target complement in the first instance. Once the ligated strand of primary probes is separated from the target strand, it will hybridize with the third and fourth probes, which can be ligated to form a complementary, secondary ligated product. It is important to realize that the ligated products are functionally equivalent to either the target or its complement. By repeated cycles of hybridization and ligation, amplification of the target sequence is achieved. A method for multiplex LCR has also been described (WO 9320227). Gap LCR (GLCR) is a version of LCR where the probes are not adjacent but are separated by 2 to 3 bases.

For amplification of mRNAs, it is within the scope of the present invention to reverse transcribe mRNA into cDNA followed by polymerase chain reaction (RT-PCR); or, to use a single enzyme for both steps as described in U.S. Patent No. 5,322,770 or, to use Asymmetric Gap LCR (RT-AGLCR) as described by Marshall et al.(1994). AGLCR is a modification of GLCR that allows the amplification of RNA.

The PCR technology is the preferred amplification technique used in the present invention. A variety of PCR techniques are familiar to those skilled in the art. For a review of PCR technology, see White (1997) and the publication entitled "PCR Methods and Applications" (1991, Cold Spring Harbor Laboratory Press). In each of these PCR procedures, PCR primers on either side of the nucleic acid sequences to be amplified are added to a suitably prepared nucleic acid sample along with dNTPs and a thermostable polymerase such as Taq polymerase, Pfu polymerase, or Vent polymerase. The nucleic acid in the sample is denatured and the PCR primers are specifically hybridized to complementary nucleic acid sequences in the sample. The hybridized primers are extended. Thereafter, another cycle of denaturation, hybridization, and extension is initiated. The cycles are repeated multiple times to produce an amplified fragment containing the nucleic acid sequence between the primer sites. PCR has further been described in several patents including US Patents 4,683,195; 4,683,202; and 4,965,188.

The PCR technology is the preferred amplification technique used to identify new biallelic markers. A typical example of a PCR reaction suitable for the purposes of the present invention is provided in Example 3.

One of the aspects of the present invention is a method for the amplification of a *TBC-1* gene, particularly the genomic sequences of SEQ ID Nos 1 and 2 or of the cDNA sequence of SEQ ID Nos 3 or 4 or a fragment or variant thereof in a test sample, preferably using the PCR technology. The method comprises the steps of contacting a test sample suspected of containing the target *TBC-1* sequence or portion thereof with amplification reaction reagents comprising a pair of amplification primers.

Thus, the present invention also relates to a method for the amplification of a *TBC-1* gene sequence, particularly of a fragment of the genomic sequence of SEQ ID No 1 or of the cDNA sequence of SEQ ID No 2 or 3, or a fragment or a variant thereof in a test sample, said method comprising the steps of :

19 biallelic markers were found in the *TBC-1* gene. They are detailed in the Table 2. They are located in intronic regions.

B- Genotyping Of *TBC-1*-Related Biallelic Markers

The polymorphisms identified above can be further confirmed and their respective frequencies can be determined through various methods using the previously described primers and probes. These methods can also be useful for genotyping either new populations in association studies or linkage analysis or individuals in the context of detection of alleles of biallelic markers which are known to be associated with a given trait. The genotyping of the biallelic markers is also important for the mapping. Those skilled in the art should note that the methods described below can be equally performed on individual or pooled DNA samples.

Once a given polymorphic site has been found and characterized as a biallelic marker as described above, several methods can be used in order to determine the specific allele carried by an individual at the given polymorphic base.

The identification of biallelic markers described previously allows the design of appropriate oligonucleotides, which can be used as probes and primers, to amplify a *TBC-1* gene containing the polymorphic site of interest and for the detection of such polymorphisms.

The biallelic markers according to the present invention may be used in methods for the identification and characterization of an association between alleles for one or several biallelic markers of the sequence of the *TBC-1* gene and a trait.

The identified polymorphisms, and consequently the biallelic markers of the invention, may be used in methods for the detection in an individual of *TBC-1* alleles associated with a trait, more particularly a trait related to a cell differentiation or abnormal cell proliferation disorders, and most particularly a trait related to cancer diseases, specifically prostate cancer.

In one embodiment the invention encompasses methods of genotyping comprising determining the identity of a nucleotide at a *TBC-1*-related biallelic marker or the complement thereof in a biological sample; optionally, wherein said *TBC-1*-related biallelic marker is selected from the group consisting of A1 to A19, and the complements thereof, or optionally the biallelic markers in linkage disequilibrium therewith; optionally, wherein said biological sample is derived from a single subject; optionally, wherein the identity of the nucleotides at said biallelic marker is determined for both copies of said biallelic marker present in said individual's genome; optionally, wherein said biological sample is derived from multiple subjects; Optionally, the genotyping methods of the invention encompass methods with any further limitation described in this disclosure, or those following, specified alone or in any combination; Optionally, said method is performed *in vitro*; optionally, further comprising amplifying a portion of said sequence comprising the biallelic marker prior to said determining step; Optionally, wherein said amplifying is performed by PCR, LCR, or replication of a recombinant vector comprising an origin of replication and said fragment in a host cell; optionally, wherein said determining is performed by a

from at least about 25 bp to 35 kbp. Amplification fragments from 25-3000 bp are typical, fragments from 50-1000 bp are preferred and fragments from 100-600 bp are highly preferred. It will be appreciated that amplification primers for the biallelic markers may be any sequence which allow the specific amplification of any DNA fragment carrying the markers. Amplification primers may be labeled or immobilized on a solid support as described in "Oligonucleotide probes and primers".

Methods of Genotyping DNA samples for Biallelic Markers

Any method known in the art can be used to identify the nucleotide present at a biallelic marker site. Since the biallelic marker allele to be detected has been identified and specified in the present invention, detection will prove simple for one of ordinary skill in the art by employing any of a number of techniques. Many genotyping methods require the previous amplification of the DNA region carrying the biallelic marker of interest. While the amplification of target or signal is often preferred at present, ultrasensitive detection methods which do not require amplification are also encompassed by the present genotyping methods. Methods well-known to those skilled in the art that can be used to detect biallelic polymorphisms include methods such as, conventional dot blot analyzes, single strand conformational polymorphism analysis (SSCP) described by Orita et al.(1989), denaturing gradient gel electrophoresis (DGGE), heteroduplex analysis, mismatch cleavage detection, and other conventional techniques as described in Sheffield et al.(1991), White et al.(1992), Grompe et al.(1989 and 1993). Another method for determining the identity of the nucleotide present at a particular polymorphic site employs a specialized exonuclease-resistant nucleotide derivative as described in US patent 4,656,127.

Preferred methods involve directly determining the identity of the nucleotide present at a biallelic marker site by sequencing assay, enzyme-based mismatch detection assay, or hybridization assay. The following is a description of some preferred methods. A highly preferred method is the microsequencing technique. The term "sequencing" is generally used herein to refer to polymerase extension of duplex primer/template complexes and includes both traditional sequencing and microsequencing.

1) Sequencing Assays

The nucleotide present at a polymorphic site can be determined by sequencing methods. In a preferred embodiment, DNA samples are subjected to PCR amplification before sequencing as described above. DNA sequencing methods are described in "Sequencing Of Amplified Genomic DNA And Identification Of Single Nucleotide Polymorphisms".

Preferably, the amplified DNA is subjected to automated dideoxy terminator sequencing reactions using a dye-primer cycle sequencing protocol. Sequence analysis allows the identification of the base present at the biallelic marker site.

2) Microsequencing Assays

In microsequencing methods, the nucleotide at a polymorphic site in a target DNA is detected by a single nucleotide primer extension reaction. This method involves appropriate microsequencing primers which, hybridize just upstream of the polymorphic base of interest in the target nucleic acid. A polymerase is used to specifically extend the 3' end of the primer with one single ddNTP (chain terminator) complementary to the nucleotide at the polymorphic site. Next the identity of the incorporated nucleotide is determined in any suitable way.

Typically, microsequencing reactions are carried out using fluorescent ddNTPs and the extended microsequencing primers are analyzed by electrophoresis on ABI 377 sequencing machines to determine the identity of the incorporated nucleotide as described in EP 412 883, the disclosure of which is incorporated herein by reference in its entirety. Alternatively capillary electrophoresis can be used in order to process a higher number of assays simultaneously. An example of a typical microsequencing procedure that can be used in the context of the present invention is provided in Example 4.

Different approaches can be used for the labeling and detection of ddNTPs. A homogeneous phase detection method based on fluorescence resonance energy transfer has been described by Chen and Kwok (1997) and Chen et al.(1997). In this method, amplified genomic DNA fragments containing polymorphic sites are incubated with a 5'-fluorescein–labeled primer in the presence of allelic dye-labeled dideoxyribonucleoside triphosphates and a modified Taq polymerase. The dye-labeled primer is extended one base by the dye-terminator specific for the allele present on the template. At the end of the genotyping reaction, the fluorescence intensities of the two dyes in the reaction mixture are analyzed directly without separation or purification. All these steps can be performed in the same tube and the fluorescence changes can be monitored in real time. Alternatively, the extended primer may be analyzed by MALDI-TOF Mass Spectrometry. The base at the polymorphic site is identified by the mass added onto the microsequencing primer.(see Haff and Smirnov, 1997).

Microsequencing may be achieved by the established microsequencing method or by developments or derivatives thereof. Alternative methods include several solid-phase microsequencing techniques. The basic microsequencing protocol is the same as described previously, except that the method is conducted as a heterogeneous phase assay, in which the primer or the target molecule is immobilized or captured onto a solid support. To simplify the primer separation and the terminal nucleotide addition analysis, oligonucleotides are attached to solid supports or are modified in such ways that permit affinity separation as well as polymerase extension. The 5' ends and internal nucleotides of synthetic oligonucleotides can be modified in a number of different ways to permit different affinity separation approaches, e.g., biotinylation. If a single affinity group is used on the oligonucleotides, the oligonucleotides can be separated from the incorporated terminator regent. This eliminates the need of physical or size separation. More than

one oligonucleotide can be separated from the terminator reagent and analyzed simultaneously if more than one affinity group is used. This permits the analysis of several nucleic acid species or more nucleic acid sequence information per extension reaction. The affinity group need not be on the priming oligonucleotide but could alternatively be present on the template. For example, immobilization can be carried out via an interaction between biotinylated DNA and streptavidin-coated microtitration wells or avidin-coated polystyrene particles. In the same manner, oligonucleotides or templates may be attached to a solid support in a high-density format. In such solid phase microsequencing reactions, incorporated ddNTPs can be radiolabeled (Syvänen, 1994) or linked to fluorescein (Livak and Hainer, 1994). The detection of radiolabeled ddNTPs can be achieved through scintillation-based techniques. The detection of fluorescein-linked ddNTPs can be based on the binding of antifluorescein antibody conjugated with alkaline phosphatase, followed by incubation with a chromogenic substrate (such as *p*-nitrophenyl phosphate). Other possible reporter-detection pairs include: ddNTP linked to dinitrophenyl (DNP) and anti-DNP alkaline phosphatase conjugate (Harju et al., 1993) or biotinylated ddNTP and horseradish peroxidase-conjugated streptavidin with *o*-phenylenediamine as a substrate (WO 92/15712). As yet another alternative solid-phase microsequencing procedure, Nyren et al.(1993) described a method relying on the detection of DNA polymerase activity by an enzymatic luminometric inorganic pyrophosphate detection assay (ELIDA).

Pastinen et al.(1997) describe a method for multiplex detection of single nucleotide polymorphism in which the solid phase minisequencing principle is applied to an oligonucleotide array format. High-density arrays of DNA probes attached to a solid support (DNA chips) are further described below.

In one aspect the present invention provides polynucleotides and methods to genotype one or more biallelic markers of the present invention by performing a microsequencing assay. Preferred microsequencing primers include the nucleotide sequences D1 to D15 and E1 to E15. It will be appreciated that the microsequencing primers listed in Example 5 are merely exemplary and that, any primer having a 3' end immediately adjacent to the polymorphic nucleotide may be used. Similarly, it will be appreciated that microsequencing analysis may be performed for any biallelic marker or any combination of biallelic markers of the present invention. One aspect of the present invention is a solid support which includes one or more microsequencing primers listed in Example 5, or fragments comprising at least 8, 12, 15, 20, 25, 30, 40, or 50 consecutive nucleotides thereof, to the extent that such lengths are consistent with the primer described, and having a 3' terminus immediately upstream of the corresponding biallelic marker, for determining the identity of a nucleotide at a biallelic marker site.

3) Mismatch detection assays based on polymerases and ligases

span the sequence segment that includes a specific polymorphism. To ensure probes that are complementary to each allele, the probes are synthesized in pairs differing at the biallelic marker. In addition to the probes differing at the polymorphic base, monosubstituted probes are also generally tiled within the detection block. These monosubstituted probes have bases at and up to a certain number of bases in either direction from the polymorphism, substituted with the remaining nucleotides (selected from A, T, G, C and U). Typically the probes in a tiled detection block will include substitutions of the sequence positions up to and including those that are 5 bases away from the biallelic marker. The monosubstituted probes provide internal controls for the tiled array, to distinguish actual hybridization from artefactual cross-hybridization. Upon completion of hybridization with the target sequence and washing of the array, the array is scanned to determine the position on the array to which the target sequence hybridizes. The hybridization data from the scanned array is then analyzed to identify which allele or alleles of the biallelic marker are present in the sample. Hybridization and scanning may be carried out as described in PCT application No. WO 92/10092 and WO 95/11995 and US patent No. 5,424,186.

Thus, in some embodiments, the chips may comprise an array of nucleic acid sequences of fragments of about 15 nucleotides in length. In further embodiments, the chip may comprise an array including at least one of the sequences selected from the group consisting of amplicons listed in table 1 and the sequences complementary thereto, or a fragment thereof, said fragment comprising at least about 8 consecutive nucleotides, preferably 10, 15, 20, more preferably 25, 30, 40, 47, or 50 consecutive nucleotides and containing a polymorphic base. In preferred embodiments the polymorphic base is within 5, 4, 3, 2, 1, nucleotides of the center of the said polynucleotide, more preferably at the center of said polynucleotide. In some embodiments, the chip may comprise an array of at least 2, 3, 4, 5, 6, 7, 8 or more of these polynucleotides of the invention. Solid supports and polynucleotides of the present invention attached to solid supports are further described in "Oligonucleotide Probes And Primers".

6) Integrated Systems

Another technique, which may be used to analyze polymorphisms, includes multicomponent integrated systems, which miniaturize and compartmentalize processes such as PCR and capillary electrophoresis reactions in a single functional device. An example of such technique is disclosed in US patent 5,589,136, which describes the integration of PCR amplification and capillary electrophoresis in chips.

Integrated systems can be envisaged mainly when microfluidic systems are used. These systems comprise a pattern of microchannels designed onto a glass, silicon, quartz, or plastic wafer included on a microchip. The movements of the samples are controlled by electric, electroosmotic or hydrostatic forces applied across different areas of the microchip to create functional microscopic valves and pumps with no moving parts.

In a further embodiment, the trait negative population can be replaced in the association studies by a random control population.

The step of testing for and detecting the presence of DNA comprising specific alleles of a biallelic marker or a group of biallelic markers of the present invention can be carried out as described further below.

Oligonucleotide Probes And Primers

The invention relates also to oligonucleotide molecules useful as probes or primers, wherein said oligonucleotide molecules hybridize specifically with a nucleotide sequence comprised in the *TBC-1* gene, particularly the *TBC-1* genomic sequence of SEQ ID Nos 1 and 2 or the *TBC-1* cDNAs sequences of SEQ ID Nos 3 and 4. More particularly, the present invention also concerns oligonucleotides for the detection of alleles of biallelic markers of the *TBC-1* gene. These oligonucleotides are useful either as primers for use in various processes such as DNA amplification and microsequencing or as probes for DNA recognition in hybridization analyses. Polynucleotides derived from the *TBC-1* gene are useful in order to detect the presence of at least a copy of a nucleotide sequence of SEQ ID Nos 1-4, or a fragment, complement, or variant thereof in a test sample.

Particularly preferred probes and primers of the invention include isolated, purified, or recombinant polynucleotides comprising a contiguous span of at least 12, 15, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 500, or 1000 nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID Nos 1 and 2, or the complements thereof. Additionally preferred probes and primers of the invention include isolated, purified, or recombinant polynucleotides comprising a contiguous span of at least 12, 15, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 500, or 1000 nucleotides of SEQ ID No 1 or the complements thereof, wherein said contiguous span comprises at least 1, 2, 3, 5, or 10 of the following nucleotide positions of SEQ ID No 1: 1-1000, 1001-2000, 2001-3000, 3001-4000, 4001-5000, 5001-6000, 6001-7000, 7001-8000, 8001-9000, 9001-10000, 10001-11000, 11001-12000, 12001-13000, 13001-14000, 14001-15000, 15001-16000, 16001-17000, and 17001-17590. Other preferred probes and primers of the invention include isolated, purified, or recombinant polynucleotides comprising a contiguous span of at least 12, 15, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 500, or 1000 nucleotides of SEQ ID No 2 or the complements thereof, wherein said contiguous span comprises at least 1, 2, 3, 5, or 10 of the following nucleotide positions of SEQ ID No 2: 1-5000, 5001-10000, 10001-15000, 15001-20000, 20001-25000, 25001-30000, 30001-35000, 35001-40000, 40001-45000, 45001-50000, 50001-55000, 55001-60000, 60001-65000, 65001-70000, 70001-75000, 75001-80000, 80001-85000, 85001-90000, 90001-95000, and 95001-99960.

Moreover, preferred probes and primers of the invention include isolated, purified, or recombinant polynucleotides comprising a contiguous span of at least 12, 15, 18, 20, 25, 30, 35, 40,

50, 60, 70, 80, 90, 100, 150, 200, 500, or 1000 nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID Nos 3 and 4, or the complements thereof.. Particularly preferred probes and primers of the invention include isolated, purified, or recombinant polynucleotides comprising a contiguous span of at least 12, 15, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 500, or 1000 nucleotides of SEQ ID No 3 or the complements thereof, wherein said contiguous span comprises at least 1, 2, 3, 5, or 10 of the following nucleotide positions of SEQ ID No 3: 1-500, 501-1000, 1001-1500, 1501-2000, 2001-2500, 2501-3000, 3001-3500, and 3501-3983. Additional preferred probes and primers of the invention include isolated, purified, or recombinant polynucleotides comprising a contiguous span of at least 12, 15, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 500, or 1000 nucleotides of SEQ ID No 4 or the complements thereof, wherein said contiguous span comprises at least 1, 2, 3, 5, or 10 of the following nucleotide positions of SEQ ID No 4: 1-500, 501-1000, 1001-1500, 1501-2000, 2001-2500, 2501-3000, 3001-3500, and 3501-3988.

Thus, the invention also relates to nucleic acid probes characterized in that they hybridize specifically, under the stringent hybridization conditions defined above, with a nucleic acid selected from the group consisting of the nucleotide sequences of SEQ ID Nos 1-4 or a variant thereof or a sequence complementary thereto.

In one embodiment the invention encompasses isolated, purified, and recombinant polynucleotides consisting of, or consisting essentially of a contiguous span of 8 to 50 nucleotides of any one of SEQ ID Nos 1 and 2 and the complement thereof, wherein said span includes a *TBC-1*-related biallelic marker in said sequence; optionally, wherein said *TBC-1*-related biallelic marker is selected from the group consisting of A1 to A19, and the complements thereof, or optionally the biallelic markers in linkage disequilibrium therewith; optionally, wherein said contiguous span is 18 to 35 nucleotides in length and said biallelic marker is within 4 nucleotides of the center of said polynucleotide; optionally, wherein said polynucleotide consists of said contiguous span and said contiguous span is 25 nucleotides in length and said biallelic marker is at the center of said polynucleotide; optionally, wherein the 3' end of said contiguous span is present at the 3' end of said polynucleotide; and optionally, wherein the 3' end of said contiguous span is located at the 3' end of said polynucleotide and said biallelic marker is present at the 3' end of said polynucleotide. In a preferred embodiment, said probes comprises, consists of, or consists essentially of a sequence selected from the following sequences: P1 to P7, P9 to P13, P15 to P19 and the complementary sequences thereto.

In another embodiment the invention encompasses isolated, purified and recombinant polynucleotides comprising, consisting of, or consisting essentially of a contiguous span of 8 to 50 nucleotides of SEQ ID Nos 1 and 2, or the complements thereof, wherein the 3' end of said contiguous span is located at the 3' end of said polynucleotide, and wherein the 3' end of said polynucleotide is located within 20 nucleotides upstream of a *TBC-1*-related biallelic marker in said

polynucleotides of the invention. One particular embodiment of these polynucleotide arrays is known as the Genechips™, and has been generally described in US Patent 5,143,854; PCT publications WO 90/15070 and 92/10092. These arrays may generally be produced using mechanical synthesis methods or light directed synthesis methods which incorporate a combination of photolithographic methods and solid phase oligonucleotide synthesis (Fodor et al., 1991). The immobilization of arrays of oligonucleotides on solid supports has been rendered possible by the development of a technology generally identified as "Very Large Scale Immobilized Polymer Synthesis" (VLSIPS™) in which, typically, probes are immobilized in a high density array on a solid surface of a chip. Examples of VLSIPS™ technologies are provided in US Patents 5,143,854; and 5,412,087 and in PCT Publications WO 90/15070, WO 92/10092 and WO 95/11995, which describe methods for forming oligonucleotide arrays through techniques such as light-directed synthesis techniques. In designing strategies aimed at providing arrays of nucleotides immobilized on solid supports, further presentation strategies were developed to order and display the oligonucleotide arrays on the chips in an attempt to maximize hybridization patterns and sequence information. Examples of such presentation strategies are disclosed in PCT Publications WO 94/12305, WO 94/11530, WO 97/29212 and WO 97/31256.

In another embodiment of the oligonucleotide arrays of the invention, an oligonucleotide probe matrix may advantageously be used to detect mutations occurring in the *TBC-1* gene and preferably in its regulatory region. For this particular purpose, probes are specifically designed to have a nucleotide sequence allowing their hybridization to the genes that carry known mutations (either by deletion, insertion or substitution of one or several nucleotides). By known mutations, it is meant, mutations on the *TBC-1* gene that have been identified according, for example to the technique used by Huang et al.(1996) or Samson et al.(1996).

Another technique that is used to detect mutations in the *TBC-1* gene is the use of a high-density DNA array. Each oligonucleotide probe constituting a unit element of the high density DNA array is designed to match a specific subsequence of the *TBC-1* genomic DNA or cDNA. Thus, an array consisting of oligonucleotides complementary to subsequences of the target gene sequence is used to determine the identity of the target sequence with the wild gene sequence, measure its amount, and detect differences between the target sequence and the reference wild gene sequence of the *TBC-1* gene. In one such design, termed 4L tiled array, is implemented a set of four probes (A, C, G, T), preferably 15-nucleotide oligomers. In each set of four probes, the perfect complement will hybridize more strongly than mismatched probes. Consequently, a nucleic acid target of length L is scanned for mutations with a tiled array containing 4L probes, the whole probe set containing all the possible mutations in the known wild reference sequence. The hybridization signals of the 15-mer probe set tiled array are perturbed by a single base change in the target sequence. As a consequence, there is a characteristic loss of signal or a "footprint" for the probes flanking a mutation position. This technique was described by Chee et al. in 1996.

(1) a genetic element or elements having a regulatory role in gene expression, for example promoters or enhancers. Enhancers are cis-acting elements of DNA, usually from about 10 to 300 bp in length that act on the promoter to increase the transcription.

(2) a structural or coding sequence which is transcribed into mRNA and eventually translated into a polypeptide, and

(3) appropriate transcription initiation and termination sequences. Structural units intended for use in yeast or eukaryotic expression systems preferably include a leader sequence enabling extracellular secretion of translated protein by a host cell. Alternatively, where a recombinant protein is expressed without a leader or transport sequence, it may include an N-terminal residue. This residue may or may not be subsequently cleaved from the expressed recombinant protein to provide a final product.

Generally, recombinant expression vectors will include origins of replication, selectable markers permitting transformation of the host cell, and a promoter derived from a highly expressed gene to direct transcription of a downstream structural sequence. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably a leader sequence capable of directing secretion of the translated protein into the periplasmic space or the extracellular medium.

The selectable marker genes for selection of transformed host cells are preferably dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, TRP1 for *S. cerevisiae* or tetracycline, rifampicin or ampicillin resistance in *E. coli*, or levan saccharase for mycobacteria.

As a representative but non-limiting example, useful expression vectors for bacterial use can comprise a selectable marker and a bacterial origin of replication derived from commercially available plasmids comprising genetic elements of pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia, Uppsala, Sweden), and GEM1 (Promega Biotec, Madison, WI, USA).

Large numbers of suitable vectors and promoters are known to those of skill in the art, and commercially available, such as bacterial vectors : pQE70, pQE60, pQE-9 (Qiagen), pbs, pD10, phagescript, psiX174, pbluescript SK, pbsks, pNH8A, pNH16A, pNH18A, pNH46A (Stratagene); ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia); or eukaryotic vectors : pWLNEO, pSV2CAT, pOG44, pXT1, pSG (Stratagene); pSVK3, pBPV, pMSG, pSVL (Pharmacia); baculovirus transfer vector pVL1392/1393 (Pharmingen); pQE-30 (QIAexpress).

A suitable vector for the expression of the TBC-1 polypeptide of SEQ ID No 5 is a baculovirus vector that can be propagated in insect cells and in insect cell lines. A specific suitable host vector system is the pVL1392/1393 baculovirus transfer vector (Pharmingen) that is used to transfect the SF9 cell line (ATCC N°CRL 1711) which is derived from *Spodoptera frugiperda*.

Other suitable vectors for the expression of the TBC-1 polypeptide of SEQ ID No 5 in a baculovirus expression system include those described by Chai et al. (1993), Vlasak et al. (1983) and Lenhard et al. (1996).

Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 viral genome, for example SV40 origin, early promoter, enhancer, splice and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

b) Promoters

The suitable promoter regions used in the expression vectors according to the present invention are chosen taking into account the cell host in which the heterologous gene has to be expressed.

A suitable promoter may be heterologous with respect to the nucleic acid for which it controls the expression or alternatively can be endogenous to the native polynucleotide containing the coding sequence to be expressed. Additionally, the promoter is generally heterologous with respect to the recombinant vector sequences within which the construct promoter/coding sequence has been inserted.

Preferred bacterial promoters are the LacI, LacZ, the T3 or T7 bacteriophage RNA polymerase promoters, the polyhedrin promoter, or the p10 protein promoter from baculovirus (Kit Novagen) (Smith et al., 1983; O'Reilly et al., 1992), the lambda P_R promoter or also the trc promoter.

Promoter regions can be selected from any desired gene using, for example, CAT (chloramphenicol transferase) vectors and more preferably pKK232-8 and pCM7 vectors. Particularly preferred bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda PR, PL and trp. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-L. Selection of a convenient vector and promoter is well within the level of ordinary skill in the art.

The choice of a promoter is well within the ability of a person skilled in the field of genetic egineering. For example, one may refer to the book of Sambrook et al. (1989) or also to the procedures described by Fuller et al. (1996).

The vector containing the appropriate DNA sequence as described above, more preferably a *TBC-1* gene regulatory polynucleotide, a polynucleotide encoding the TBC-1 polypeptide of SEQ ID No 5 or both of them, can be utilized to transform an appropriate host to allow the expression of the desired polypeptide or polynucleotide.

present invention is the human adenovirus type 2 or 5 (Ad 2 or Ad 5) or an adenovirus of animal origin (French patent application N° FR-93.05954).

Retrovirus vectors and adeno-associated virus vectors are generally understood to be the recombinant gene delivery systems of choice for the transfer of exogenous polynucleotides *in vivo* , particularly to mammals, including humans. These vectors provide efficient delivery of genes into cells, and the transferred nucleic acids are stably integrated into the chromosomal DNA of the host

Particularly preferred retroviruses for the preparation or construction of retroviral *in vitro* or *in vitro* gene delivery vehicles of the present invention include retroviruses selected from the group consisting of Mink-Cell Focus Inducing Virus, Murine Sarcoma Virus, Reticuloendotheliosis virus and Rous Sarcoma virus. Particularly preferred Murine Leukemia Viruses include the 4070A and the 1504A viruses, Abelson (ATCC No VR-999), Friend (ATCC No VR-245), Gross (ATCC No VR-590), Rauscher (ATCC No VR-998) and Moloney Murine Leukemia Virus (ATCC No VR-190; PCT Application No WO 94/24298). Particularly preferred Rous Sarcoma Viruses include Bryan high titer (ATCC Nos VR-334, VR-657, VR-726, VR-659 and VR-728). Other preferred retroviral vectors are those described in Roth et al. (Roth J.A. et al., 1996), PCT Application No WO 93/25234, PCT Application No WO 94/ 06920, Roux et al., 1989, Julan et al., 1992 and Neda et al., 1991.

Yet another viral vector system that is contemplated by the invention consists in the adeno-associated virus (AAV). The adeno-associated virus is a naturally occurring defective virus that requires another virus, such as an adenovirus or a herpes virus, as a helper virus for efficient replication and a productive life cycle (Muzyczka et al., 1992). It is also one of the few viruses that may integrate its DNA into non-dividing cells, and exhibits a high frequency of stable integration (Flotte et al., 1992; Samulski et al., 1989; McLaughlin et al., 1989). One advantageous feature of AAV derives from its reduced efficacy for transducing primary cells relative to transformed cells.

Other compositions containing a vector of the invention advantageously comprise an oligonucleotide fragment of a nucleic sequence selected from the group consisting of SEQ ID Nos 3 or 4 as an antisense tool that inhibits the expression of the corresponding *TBC-1* gene. Preferred methods using antisense polynucleotide according to the present invention are the procedures described by Sczakiel et al. (1995) or those described in PCT Application No WO 95/24223.

Host cells

Another object of the invention consists in host cell that have been transformed or transfected with one of the polynucleotides described therein, and more precisely a polynucleotide either comprising a *TBC-1* regulatory polynucleotide or the coding sequence of the TBC-1 polypeptide having the amino acid sequence of SEQ ID No 5. Are included host cells that are transformed (prokaryotic cells) or that are transfected (eukaryotic cells) with a recombinant vector such as one of those described above.

Screening Of Agents Interacting With TBC-1

In a further embodiment, the present invention also concerns a method for the screening of new agents, or candidate substances interacting with TBC-1. These new agents could be useful against cancer.

In a preferred embodiment, the invention relates to a method for the screening of candidate substances comprising the following steps:

- providing a cell line, an organ, or a mammal expressing a *TBC-1* gene or a fragment thereof, preferably the regulatory region or the promoter region of the *TBC-1* gene.

- obtaining a candidate substance preferably a candidate substance capable of inhibiting the binding of a transcription factor to the *TBC-1* regulatory region,

- testing the ability of the candidate substance to decrease the symptoms of prostate cancer and/or to modulate the expression levels of *TBC-1*.

In some embodiments, the cell line, organ or mammal expresses a heterologous protein, the coding sequence of which is operably linked to the *TBC-1* regulatory or promoter sequence. In other embodiments, they express a *TBC-1* gene comprising alleles of one or more *TBC-1*-related biallelic markers.

A candidate substance is a substance which can interact with or modulate, by binding or other intramolecular interactions, expression, stability, and function of *TBC-1*. Such substances may be potentially interesting for patients who are not responsive to existing drugs or develop side effects to them. Screening may be effected using either *in vitro* methods or *in vivo* methods.

Such methods can be carried out in numerous ways such as on transformed cells which express the considered alleles of the *TBC-1* gene, on tumors induced by said transformed cells, for example in mice, or on a TBC-1 protein encoded by the considered allelic variant of TBC-1.

Screening assays of the present invention generally involve determining the ability of a candidate substance to present a cytotoxic effect, to change the characteristics of transformed cells such as proliferative and invasive capacity, to affect the tumor growth, or to modify the expression level of TBC-1.

Typically, this method includes preparing transformed cells with different forms of *TBC-1* sequences containing particular alleles of one or more biallelic markers and/or trait causing mutations described above. This is followed by testing the cells expressing the TBC-1 with a candidate substance to determine the ability of the substance to present cytotoxic effect, to affect the characteristics of transformed cells, the tumor growth, or to modify the expression level of *TBC-1*.

Typical examples of such drug screening assays are provided below. It is to be understood that the parameters set forth in these examples can be modified by the skilled person without undue experimentation.

Methods for screening substances interacting with a TBC-1 polypeptide

A method for the screening of a candidate substance according to the invention comprises the following steps :

a)providing a polypeptide comprising the amino acid sequence SEQ ID No 5, or a peptide fragment or a variant thereof;

b) obtaining a candidate substance;

c) bringing into contact said polypeptide with said candidate substance;

d) detecting the complexes formed between said polypeptide and said candidate substance.

For the purpose of the present invention, a ligand means a molecule, such as a protein, a peptide, an antibody or any synthetic chemical compound capable of binding to the TBC-1 protein or one of its fragments or variants or to modulate the expression of the polynucleotide coding for TBC-1 or a fragment or variant thereof.

In the ligand screening method according to the present invention, a biological sample or a defined molecule to be tested as a putative ligand of the TBC-1 protein is brought into contact with a purified TBC-1 protein, for example a purified recombinant TBC-1 protein produced by a recombinant cell host as described hereinbefore, in order to form a complex between the TBC-1 protein and the putative ligand molecule to be tested.

A. Candidate ligands obtained form random peptide libraries

In a particular embodiment of the screening method, the putative ligand is the expression product of a DNA insert contained in a phage vector (Parmley and Smith, 1988). Specifically, random peptide phages libraries are used. The random DNA inserts encode peptides of 8 to 20 aminoacids in length (Oldenburg K.R. et al., 1992,.; Valadon P., et al., 1996; Lucas A.H., 1994; Westerink M.A.J., 1995; Castagnoli L. et al., 1991). According to this particular embodiment, the recombinant phages expressing a protein that binds to the immobilized TBC-1 protein are retained and the complex formed between the TBC-1 protein and the recombinant phage may be subsequently immunoprecipitated by a polyclonal or a monoclonal antibody directed against the TBC-1 protein.

Once the ligand library in recombinant phages has been constructed, the phage population is brought into contact with the immobilized TBC-1 protein. Then the preparation of complexes is washed in order to remove the non-specifically bound recombinant phages. The phages that bind specifically to the TBC-1 protein are then eluted by a buffer (acid pH) or immunoprecipitated by the anti-TBC-1monoclonal antibody produced by a hybridoma, and this phage population is subsequently amplified by an over-infection of bacteria (for example E. coli). The selection step may be repeated several times, preferably 2-4 times, in order to select the more specific recombinant phage clones. The last step consists in characterizing the peptide produced by the selected recombinant phage clones either by expression in infected bacteria and isolation,

Expression levels and patterns of *TBC-1* may be analyzed by solution hybridization with long probes as described in International Patent Application No. WO 97/05277, the entire contents of which are incorporated herein by reference. Briefly, the *TBC-1* cDNA or the *TBC-1* genomic DNA described above, or fragments thereof, is inserted at a cloning site immediately downstream of a bacteriophage (T3, T7 or SP6) RNA polymerase promoter to produce antisense RNA. Preferably, the *TBC-1* insert comprises at least 100 or more consecutive nucleotides of the genomic DNA sequence or the cDNA sequences, particularly those comprising one of the nuceotide sequences of SEQ ID Nos 3, 4 and 6-8 or those encoding a mutated TBC-1. The plasmid is linearized and transcribed in the presence of ribonucleotides comprising modified ribonucleotides (i.e. biotin-UTP and DIG-UTP). An excess of this doubly labeled RNA is hybridized in solution with mRNA isolated from cells or tissues of interest. The hybridizations are performed under standard stringent conditions (40-50°C for 16 hours in an 80% formamide, 0.4 M NaCl buffer, pH 7-8). The unhybridized probe is removed by digestion with ribonucleases specific for single-stranded RNA (i.e. RNases CL3, T1, Phy M, U2 or A). The presence of the biotin-UTP modification enables capture of the hybrid on a microtitration plate coated with streptavidin. The presence of the DIG modification enables the hybrid to be detected and quantified by ELISA using an anti-DIG antibody coupled to alkaline phosphatase.

Quantitative analysis of *TBC-1* gene expression may also be performed using arrays. As used herein, the term array means a one dimensional, two dimensional, or multidimensional arrangement of a plurality of nucleic acids of sufficient length to permit specific detection of expression of mRNAs capable of hybridizing thereto. For example, the arrays may contain a plurality of nucleic acids derived from genes whose expression levels are to be assessed. The arrays may include the *TBC-1* genomic DNA, the *TBC-1* cDNA sequences or the sequences complementary thereto or fragments thereof, particularly those comprising at least one of the biallelic markers according the present invention. Preferably, the fragments are at least 15 nucleotides in length. In other embodiments, the fragments are at least 25 nucleotides in length. In some embodiments, the fragments are at least 50 nucleotides in length. More preferably, the fragments are at least 100 nucleotides in length. In another preferred embodiment, the fragments are more than 100 nucleotides in length. In some embodiments the fragments may be more than 500 nucleotides in length.

For example, quantitative analysis of *TBC-1* gene expression may be performed with a complementary DNA microarray as described by Schena et al. (1995). Full length *TBC-1* cDNAs or fragments thereof are amplified by PCR and arrayed from a 96-well microtiter plate onto silylated microscope slides using high-speed robotics. Printed arrays are incubated in a humid chamber to allow rehydration of the array elements and rinsed, once in 0.2% SDS for 1 min, twice in water for 1 min and once for 5 min in sodium borohydride solution. The arrays are submerged in water for 2 min at 95°C, transferred into 0.2% SDS for 1 min, rinsed twice with water, air dried and stored in the dark at 25°C.

b) obtaining a candidate substance, and

c) determining the ability of the candidate substance to modulate the expression levels of the polynucleotide encoding the detectable protein.

In a preferred embodiment of the above screening method, the nucleic acid comprising the 5' regulatory region sequence or a biologically active fragment or variant thereof also includes a 5'UTR region of one of the *TBC-1* cDNAs of SEQ ID Nos 3 and 4, or one of their biologically active fragments or variants thereof.

A second method for the screening of a candidate substance or molecule that modulates the expression of the *TBC-1* gene comprises the following steps :

a) providing a recombinant cell host containing a nucleic acid, wherein said nucleic acid comprises a 5'UTR sequence of one of the *TBC-1* cDNAs of SEQ ID Nos 3 and 4, or one of their biologically active fragments or variants, the 5'UTR sequence or its biologically active fragment or variant being operably linked to a polynucleotide encoding a detectable protein;

b) obtaining a candidate substance, and

c) determining the ability of the candidate substance to modulate the expression levels of the polynucleotide encoding the detectable protein.

In a preferred embodiment of the screening method described above, the nucleic acid that comprises a nucleotide sequence selected from the group consisting of the 5'UTR sequence of one of the *TBC-1* cDNAs of SEQ ID Nos 3 and 4 or one of their biologically active fragments or variants, includes a promoter sequence, wherein said promoter sequence can be either endogenous, or in contrast exogenous with respect to the *TBC-1* 5'UTR sequences defined therein.

Among the preferred polynucleotides encoding a detectable protein, there may be cited polynucleotides encoding beta galactosidase, green fluorescent protein (GFP) and chloramphenicol acetyl transferase (CAT).

For the design of suitable recombinant vectors useful for performing the screening methods described above, it will be referred to the section of the present specification wherein the preferred recombinant vectors of the invention are detailed.

Screening using transgenic animals

In vivo methods can utilize transgenic animals for drug screening. Nucleic acids including at least one of the biallelic polymorphisms of interest can be used to generate genetically modified non-human animals or to generate site specific gene modifications in cell lines. The term "transgenic" is intended to encompass genetically modified animals having a deletion or other knock-out of *TBC-1* gene activity, having an exogenous *TBC-1* gene that is stably transmitted in the host cells, or having an exogenous *TBC-1* promoter operably linked to a reporter gene. Transgenic animals may be made through homologous recombination, where the *TBC-1* locus is altered. Alternatively, a nucleic acid construct is randomly integrated into the genome. Vectors for stable integration include for example plasmids, retroviruses and other animal viruses, and YACs. Of

interest are transgenic mammals e.g. cows, pigs, goats, horses, and particularly rodents such as rats and mice. Transgenic animals allow to study both efficacy and toxicity of the candidate drug.

Methods for inhibiting the expression of a *TBC-1* gene

Other therapeutic compositions according to the present invention comprise advantageously an oligonucleotide fragment of the nucleic sequence of *TBC-1* as an antisense tool that inhibits the expression of the corresponding *TBC-1* gene. Preferred methods using antisense polynucleotide according to the present invention are the procedures described by Sczakiel et al. (1995).

Preferably, the antisense tools are chosen among the polynucleotides (15-200 bp long) that are complementary to the 5'end of the *TBC-1* mRNA. In another embodiment, a combination of different antisense polynucleotides complementary to different parts of the desired targetted gene are used.

Preferred antisense polynucleotides according to the present invention are complementary to a sequence of the mRNAs of *TBC-1* that contains the translation initiation codon ATG.

The antisense nucleic acid molecules to be used in gene therapy may be either DNA or RNA sequences. They comprise a nucleotide sequence complementary to the targeted sequence of the PTCA-1 genomic DNA, the sequence of which can be determined using one of the detection methods of the present invention. The targeted DNA or RNA sequence preferably comprises at least one of the biallelic markers according to the present invention. The antisense nucleic acids should have a length and melting temperature sufficient to permit formation of an intracellular duplex having sufficient stability to inhibit the expression of the *TBC-1* mRNA in the duplex. Strategies for designing antisense nucleic acids suitable for use in gene therapy are disclosed in Green et al., (1986) and Izant and Weintraub, (1984), the disclosures of which are incorporated herein by reference.

In some strategies, antisense molecules are obtained by reversing the orientation of the *TBC-1* coding region with respect to a promoter so as to transcribe the opposite strand from that which is normally transcribed in the cell. The antisense molecules may be transcribed using in vitro transcription systems such as those which employ T7 or SP6 polymerase to generate the transcript. Another approach involves transcription of *TBC-1* antisense nucleic acids in vivo by operably linking DNA containing the antisense sequence to a promoter in a suitable expression vector.

Alternatively, suitable antisense strategies are those described by Rossi et al. (1991), in the International Applications Nos. WO 94/23026, WO 95/04141, WO 92/18522 and in the European Patent Application No. EP 0 572 287 A2

An alternative to the antisense technology that is used according to the present invention consists in using ribozymes that will bind to a target sequence via their complementary polynucleotide tail and that will cleave the corresponding RNA by hydrolyzing its target site (namely « hammerhead ribozymes »). Briefly, the simplified cycle of a hammerhead ribozyme consists of (1) sequence specific binding to the target RNA via complementary antisense sequences;

99-20481	A18	99-20481-419	Intron I	A	T	84810
99-20480	A19	99-20480-233	Intron J	A	G	89967

BM refers to "biallelic marker". All1 and all2 refer respectively to allele 1 and allele 2 of the biallelic marker.

Table 3

BM	Marker Name	Position range of probes in SEQ ID No 1		Probes
A1	99-430-352	9482	9506	P1
BM	**Marker Name**	**Position range of probes in SEQ ID No 2**		**Probes**
A2	99-20508-456	1431	1455	P2
A3	99-20469-213	5235	5259	P3
A4	5-254-227	6211	6235	P4
A5	5-257-353	14711	14735	P5
A6	99-20511-32	19174	19198	P6
A7	99-20511-221	18985	19009	P7
A9	99-20504-90	29605	29629	P9
A10	99-20493-238	42507	42531	P10
A11	99-20499-221	69312	69336	P11
A12	99-20499-364	69169	69193	P12
A13	99-20499-399	69134	69158	P13
A15	5-249-304	78583	78607	P15
A16	99-20485-269	82147	82171	P16
A17	99-20481-131	84510	84534	P17
A18	99-20481-419	84798	84822	P18
A19	99-20480-233	89955	89979	P19

Example 5 :

Validation of the polymorphisms through microsequencing

The biallelic markers identified in example 4 were further confirmed and their respective frequencies were determined through microsequencing. Microsequencing was carried out for each individual DNA sample described in Example 2.

Amplification from genomic DNA of individuals was performed by PCR as described above for the detection of the biallelic markers with the same set of PCR primers (Table 1).

The preferred primers used in microsequencing were about 19 nucleotides in length and hybridized just upstream of the considered polymorphic base. According to the invention, the primers used in microsequencing are detailed in Table 4.

Table 4

Marker Name	Biallelic Marker	Mis. 1	Position range of microsequencing	Mis. 2	Complementary position range of

on their position. When two significant peaks are detected for the same position, each sample is categorized classification as homozygous or heterozygous type based on the height ratio.

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AMENDMENT UNDER 37 C.F.R. §1.116
Examining Group 1634
Patent Application
Docket No. G-046US02PCT
Serial No. 09/762,311

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Carla J. Myers
Art Unit	:	1634
Applicants	:	Marta Blumenfeld, Lydie Bougueleret, Ilya Chumakov
Serial No.	:	09/762,311
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For	:	Nucleic Acids Encoding Human TBC-1 Protein and Polymorphic Markers Thereof

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AMENDMENT UNDER 37 C.F.R. § 1.116

Sir:

In response to the Office Action dated February 9, 2004, please amend the above-identified application as follows:

In the Claims

1-49. (Canceled)

50. (Currently Amended) A composition comprising: an isolated, purified, or recombinant polynucleotide which encodes a polypeptide comprising ~~a contiguous span of at least 6 amino acids of~~ SEQ ID NO:5 or the complement thereof, provided that said polypeptide is not murine TBC-1.

51-64. (Canceled)

65. (Currently Amended) A composition comprising an isolated, purified, or recombinant human polynucleotide which encodes a polypeptide comprising ~~a contiguous span of at least 6 amino acids of~~ SEQ ID NO:5 or the complement thereof.

66-73. (Canceled)

74. (New) An isolated, purified, or recombinant polynucleotide which: a) encodes a polypeptide comprising SEQ ID NO: 5; b) comprises nucleotides 171 to 3725 of SEQ ID NO: 3; or c) the complement thereof.

75. (New) The isolated, purified, or recombinant polynucleotide according to claim 74 attached to a solid support.

76. (New) The isolated, purified, or recombinant polynucleotide according to claim 75, wherein said polynucleotide encodes a polypeptide comprising SEQ ID NO: 5.

77. (New) The isolated, purified, or recombinant polynucleotide according to claim 75, wherein said polynucleotide comprises nucleotides 171 to 3725 of SEQ ID NO: 3.

78. (New) The isolated, purified, or recombinant polynucleotide according to claim 74, wherein said polynucleotide encodes a polypeptide comprising SEQ ID NO: 5.

79. (New) The isolated, purified, or recombinant polynucleotide according to claim 74, wherein said polynucleotide comprises nucleotides 171 to 3725 of SEQ ID NO: 3.

80. (New) An array of polynucleotides comprising at least one isolated, purified, or recombinant polynucleotide which: a) encodes a polypeptide comprising SEQ ID NO: 5; b) comprises nucleotides 171 to 3725 of SEQ ID NO: 3; or c) the complement thereof..

81. (New) The array according to claim 80, wherein said polynucleotide encodes a polypeptide comprising SEQ ID NO: 5.

82. (New) The array according to claim 80, wherein said polynucleotide comprises nucleotides 171 to 3725 of SEQ ID NO: 3.

83. (New) The array according to claim 80, wherein said array is addressable.

84. (New) The array according to claim 81, wherein said array is addressable.

85. (New) The array according to claim 82, wherein said array is addressable.

86. (New) The isolated, purified, or recombinant polynucleotide according to claim 76, further comprising a label.

87. (New) The isolated, purified, or recombinant polynucleotide according to claim 77, further comprising a label.

88. (New) A composition comprising: a recombinant vector comprising a polynucleotide which: a) encodes a polypeptide comprising SEQ ID NO: 5; b) comprises nucleotides 171 to 3725 of SEQ ID NO: 3; or c) the complement thereof.

89. (New) The composition according to claim 88, wherein said recombinant vector comprises a polynucleotide which encodes a polypeptide comprising SEQ ID NO: 5.

90. (New) The composition according to claim 88, wherein said recombinant vector comprises nucleotides 171 to 3725 of SEQ ID NO: 3.

91. (New) A composition comprising: a host cell comprising a recombinant vector comprising a polynucleotide which: a) encodes a polypeptide comprising SEQ ID NO: 5; b) comprises nucleotides 171 to 3725 of SEQ ID NO: 3; or c) the complement thereof.

92. (New) The composition according to claim 91, wherein said recombinant vector comprises a polynucleotide which encodes a polypeptide comprising SEQ ID NO: 5.

93. (New) The composition according to claim 91, wherein said recombinant vector comprises nucleotides 171 to 3725 of SEQ ID NO: 3.

94. (New) A method of making a TBC-1 polypeptide comprising the steps of:

(i) obtaining a host cell comprising a recombinant vector comprising a polynucleotide which: a) encodes a polypeptide comprising SEQ ID NO: 5 or b) comprises nucleotides 171 to 3725 of SEQ ID NO: 3.

(ii) growing said cell under conditions suitable to produce said polypeptide.

95. (New) The method according to claim 94, wherein said recombinant vector comprises a polynucleotide which encodes a polypeptide comprising SEQ ID NO: 5.

96. (New) The method according to claim 94, wherein said recombinant vector comprises nucleotides 171 to 3725 of SEQ ID NO: 3.

97. (New) The method according to claim 95, further comprising the step of purifying or isolating said polypeptide.

98. (New) The method according to claim 96, further comprising the step of purifying or isolating said polypeptide.

Remarks

Claims 42, 43, and 45-73 are pending in the subject application. By way of the amendment of this date, claims 74-98 have been added, claims 42, 43, 45-49, 51-64, and 66-73 have been canceled, and claims 50 and 65 have been amended. Certain of the claims have been amended for the purpose of expediting the patent application process in a manner consistent with the Patent and Trademark Office Patent Business Goals (PBG), 65 Fed. Reg. 54603 (September 8, 2000), in order to advance prosecution and facilitate the business interests of Applicant(s). Support for the claims and the amendments to the pending claims can be found throughout the subject specification (including, for example, the originally presented claims, the sequence listing at pages 43-51, and the specification at page 23, lines 23-25, page 28, lines 1-23, and page 53, line 9 through page 62, line 24). Favorable consideration of the claims now presented, in view of the remarks and amendments set forth herein, is earnestly solicited.

Applicants note that the Examiner has indicated that claims 66-67 were objected to as being dependent upon a rejected base claim, but being allowable upon being rewritten in independent form, including all limitations of the base claim and any intervening claim. Applicants have amended claims 50 and 65 to conform with the indicated allowable subject matter and respectfully submit that the claims are now in condition for allowance.

Applicants have also presented additional new claims directed to vectors, host cells, and arrays comprising the polynucleotides indicated as being allowable in the previous Office Action. Method of use claims, directed to the production of *TBC-1* polypeptides, comprising obtaining a host cell comprising a recombinant vector comprising a polynucleotide (previously indicated as being allowable) and growing the host cell under conditions that allow for the production of TBC-1 polypeptide have also been presented. It is respectfully submitted that the newly presented claims do not require new consideration and do not raise an issue of new matter.

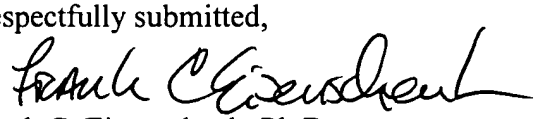
Applicants respectfully submit that the previous rejections of record are moot in view of the cancellation of the rejected claims. However, it should be understood that the amendments presented herein have been made solely to expedite prosecution of the subject application to completion and should not be construed as an indication of Applicants' agreement with or acquiescence in the Examiner's position. Applicants expressly reserve the right to pursue the invention(s) disclosed in

the subject application, including any subject matter canceled or not pursued during prosecution of the subject application, in a related application.

In view of the foregoing remarks and the amendments to the claims, the applicants believe that the pending claims are now in condition for allowance, and such action is respectfully requested. The Commissioner is hereby authorized to charge any fees under 37 C.F.R. 1.16 or 1.17 as required by this paper to Deposit Account 19-0065.

Applicants also invite the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephone interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,



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CERTIFICATE OF CORRECTION

PATENT NO. : 6,825,004

DATED : November 30, 2004

INVENTORS : Marta Blumenfeld, Lydie Bougueleret, Ilya Chumakov

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